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Splicing to keep cycling: The importance of pre-mRNA splicing during the cell cycle

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Abstract

Pre-mRNA splicing is a fundamental process in mammalian gene expression and alternative splicing plays an extensive role in generating protein diversity. Since the majority of genes undergo pre-mRNA splicing, most cellular processes depend on proper spliceosome function. Here we focus on the cell cycle and describe its dependence on pre-mRNA splicing and accurate alternative splicing. We outline the key cell cycle factors and their known alternative splicing isoforms. We discuss different levels of pre-mRNA splicing regulation such as post-translational modifications and changes in expression of splicing factors. We describe the effect of chromatin dynamics on pre-mRNA splicing during the cell cycle. In addition, we focus on the spliceosome component SF3B1, which is mutated in many types of cancer, and describe the link of SF3B1 and its inhibitors to cell cycle.

Pre-mRNA splicing occurs in 97% of human genes. This suggests that most if not all cellular processes are dependent on the splicing machinery. Here we focus on the cell cycle process and discuss its many connections with pre-mRNA splicing. Splicing is regulated by both splicing factors and chromatin, which are known to alter during the cell cycle. We will review the changes in these two regulators and their effect on cell cycle.

Cell cycle

The cell-cycle process is highly conserved and precisely controlled to conduct genome replication. The process consists of four distinct phases, G0/G1, S (DNA synthesis), G2, and M (mitosis) with multiple checkpoints. These cell cycle checkpoints ensure reliable replication of the cell's DNA in the S phase and the exact division of the chromosomes into daughter cells. Ensuring that all chromosomes are aligned correctly on the mitotic spindle before division of the two daughter cells prevents dead, aneuploid, or mutant cells. The G1 and G2 phases serve as gaps between the S and M phases. G1 is the point where the decision is made whether to go into S phase and replicate the DNA or to exit the cell cycle and go to G0. G2 ensures full replication of the genome in S phase and the transition to cell division in M phase.

Cell cycle decisions are made by assessing both intra- and extracellular signals. Intracellular signals are proper replication of the DNA and the correct post-translational modification pattern of cell cycle proteins. Those proteins are cyclins and cyclin-dependent kinases (CDKs), which are a group of serine/threonine kinases. They form complexes with cyclins to stabilize, activate, and phosphorylate key cell cycle factors at specific phases [1]. CDKs' catalytic activities are regulated by interactions with cyclins and CDK inhibitors (CKIs). The formation of cyclin/CDK complexes controls the cell-cycle progression via phosphorylation of the target genes [2].

Epigenetic dynamics during cell-cycle progression

Chromatin binding proteins and chromatin modifications are dynamic during the cell cycle and chromatin impacts cell cycle events such as origin firing, chromosome segregation at mitosis and gene expression. Chromatin dynamics stem from histone modifiers which fluctuate in expression and post-translational modifications in a cell cycle-dependent manner [3]. For example, CDK1 and CDK2 can phosphorylate the chromatin factor, enhancer of zeste homolog 2 (EZH2), in a cell cycle-dependent manner and in turn silence genes during the cell cycle [4]. Chromatin also plays a large role in correct chromosome segregation by allowing chromosomes to condensate. This condensation is enabled by phosphorylation of H3 by the

Aurora B kinase that recruits the Condensin I complexes [5]. In addition, phosphorylation of H3 modulates the binding of repressive chromatin proteins to mitotic chromosomes [6].

Gene expression during cell-cycle progression

Many proteins that carry out important functions during the cell cycle display a cyclical expression pattern that is often regulated at the transcriptional level [7-9]. Thus, genes expressed in specific phases of the cell cycle are different from those expressed at steady-state [10]. Sensitive methods that identify small amounts of mRNA have identified specific transcripts expressed at each phase. These methods have also helped identify **enhancer RNA** (see Glossary) which indicates the specific enhancers activated during the cell cycle [10]. This adjusted expression requires a well-orchestrated regulation that also needs to adapt to the changing amount of DNA in the cell. This is made possible by regulation via chromatin structure. In addition to change in gene expression, M phase is considered to have less transcription and translation in general [11]. Interestingly cell-free lysates from mitotic cells are also less efficient in *in vitro* pre-mRNA splicing [11].

Pre-mRNA splicing

97% of human genes harbor introns which are removed in a process called pre-mRNA splicing [12]. The splicing reaction is catalyzed by the spliceosome, a multi-subunit complex, comprised of proteins and small noncoding RNAs (U1,U2, U4, U5 and U6) [13-15]. The spliceosome coordinates two transesterification reactions necessary to remove introns and to join the adjacent exons. It functions by step-wise formation of sub-complexes that recognize motif sequences on the pre-mRNA and promote efficient splicing [16].

Adjacent exons are not constitutively spliced together; alternative splicing is a process by which specific exons are selectively included or excluded [14]. Alternative splicing occurs in more than 90% of genes and allows for the production of multiple protein isoforms from a single pre-mRNA molecule by the use of splice sites [17-19]. Each isoform produced can harbor different functions and so alternative splicing contributes significantly to regulation of cellular function [20]. The main forms of alternative splicing are exon skipping, intron retention, alternative 3′ splice site, alternative 5′ splice site, and mutually exclusive exons [21]. In addition to protein diversity, alternative splicing can also lead to reduced translation of an mRNA by introduction of a toxic exon or intron which gives rise to a transcript with a premature stop codon that might undergo nonsense-mediated mRNA decay (NMD) or a transcript that will be sequestered in the nucleus [22].

Alternative splicing is frequently regulated by trans-acting factors binding to sequence motifs that are associated with the enhancing or silencing of pre-mRNA splicing. These motifs can be found in either exons or introns [13]. They are bound by an array of RNA-binding proteins that can be divided into two groups; the serine/arginine-rich proteins (SR proteins) and a heterogeneous group of nuclear ribonucleoproteins (hnRNPs) [23]. SR proteins harbor one or two RNA recognition motif (RRM) domains at the amino terminus that allow for RNA-binding specificity and a carboxy-terminal arginine/serine-rich (RS) domain affecting protein–protein interactions [24]. hnRNPs harbor the RRM domains and **unstructured domains** that could regulate their protein–protein interactions [25].

A major study of pre-mRNA splicing during cell cycle was conducted in human cervical cancer cells by RNA-seq analysis of synchronously dividing HeLa cells [26]. Dominguez *et al*. discovered 1182 genes whose gene expression was altered during the cell cycle and 1293 genes whose alternative splicing changed during the cell cycle and there was a minor overlap between these two groups [26]. This study suggested independent regulation of the two processes during cell cycle. Of all the genes expressed in the HeLa cells studied, 15% had periodic cell cycle-dependent alternative splicing. In addition to a major class of alternative splicing of cassette exons, sequencing RNA also pointed to intron retention as a key event regulated during cell cycle [26].

Since almost all genes undergo splicing it is reasonable to assume that cell cycle progression, or any other cell process, will be dependent on spliceosome function. Evidence that strengthens this assumption come from the results of multiple screens searching for novel cell cycle genes, such as those of Hwang *et al*. in yeast and Neumann *et al*. in HeLa cells [27, 28]. These screens led to the prediction that arrest is a general consequence of interfering with core spliceosome function; the genes revealed as cell cycle genes in these and further studies are *DHX8* [27], *PRPF8* [29, 30], *U2AF2* [31], *CDC5L* [32, 33], *DDX46* and *PRPF6* [34].

Since splicing is an essential process in mammalian cells, it can affect the ability of cells to proliferate by giving rise to a specific alternative spliced isoform [35] and the generation of specific splicing isoforms has recently been found to act as a driver of cancer [35-39] and can inhibit apoptosis (reviewed in [40]). In addition, global alterations in splicing arrays in cancer can be the result of changes in expression of splicing factors that may dictate an oncogenic splicing pattern [13–16], or by mutations that give rise to a specific splicing isoform with a potential to promote cancer [12]. In this review we focus on pre-mRNA splicing that affects the cell cycle directly and thus will not cover proliferation or apoptosis. An additional role for

some splicing factors is resolving **R loops**, generated between nascent transcripts and the DNA while transcription take place. Problems with R loop resolution lead to DNA damage and as a result halt cell cycle. This indirect effect of splicing factors on cell cycle is also not part of this review (reviewed in [41]). Many splicing factors have also been found to have additional functions in mitosis that do not involve pre-mRNA splicing (reviewed in [42]). Here we will focus on two main intersections between pre-mRNA splicing and cell cycle. The first is the need for pre-mRNA splicing to allow for production of key factors of the cell cycle that harbor introns. The second is alternative splicing of those key factors in order to produce the specific isoform needed for cell cycle progression. In addition, we will detail what is known to date about the role of splicing factors in the process that occur during the cell cycle. These splicing factors can be regulated in several ways: 1) by post-translational modifications; 2) at the level of their expression; 3) and via chromatin. We will review all of these different aspects here.

Pre-mRNA splicing of cell cycle key factors

Cell division cycle 25 (CDC25): Currently the only known targets of the members of the CDC25 family of phosphatases, CDC25A, B and C, are CDK/cyclin complexes [43, 44]. This function sets CDC25 as a regulator of all cell cycle stages, as its targets are CDK2/cyclin E at the G1–S transition and CDK1–cyclin B at the entry into mitosis [45]. Regulation of the CDC25 family was found to be at the protein level but also via alternative splicing [46-50]. Not much is known about the role of each of the alternative splicing isoforms or their regulation, but data exist on the change in phosphatase function [51] and differential protein stability between CDC25B isoforms [52]. In addition, U2AF1 was shown to be important for the pre-mRNA splicing of both CDC25B and C [53]. Andersen *et al*. identified Prp38 or Mfap1 as regulators of CDC25 (named mitotic phosphatase string) in *Drosophila* cells [54]. Depletion of PRP38 or MFAP1 led to arrest in G2/M, which might be a result of abnormal splicing of the CDC25.

Aurora kinase B (AURKB): AURKB is a member of the aurora kinase subfamily of serine/threonine kinases, part of the **chromosomal passenger complex (CPC)**, and is required for chromatin-induced microtubule stabilization and spindle assembly. While AURKB has several coding alternative splicing isoforms, their regulation during the cell cycle is not known. Lately it was suggested that an intron retention isoform with a premature termination codon is promoted during S/G2. As a result, nonsense-mediated decay of this isoform leads to reduced AURKB protein amount following M phase [26].

CDC-like kinase 1 (CLK1): CLK1's role as a kinase of splicing factors will be detailed in the next section. Alternative splicing regulation of CLK1 exon EB was shown to be important for its kinase activity [55, 56]. Inclusion of exon EB promotes a catalytically active CLK1. Interestingly CLK1 auto-regulates its alternative splicing, as the CLK1 isoform with exon EB promotes exon EB exclusion while the CLK1 isoform without exon EB promotes its inclusion [55, 56].

CDK2: CDK2 is a serine/threonine protein kinase, which has a role in all cell cycle phases. CDK2/cyclin E complex contributes to the phosphorylation of the retinoblastoma (RB) protein which drives the cell to S phase (Figure 1). In late S phase CDK2/cyclin A phosphorylates different targets to control the cell cycle transition from S to G2. For example, CDK2/cyclin A phosphorylates CDC6, an initiator of DNA replication. Phosphorylated CDC6 translocates to the cytoplasm to prevent re-replication of the DNA [57] (Figure 1). Regulation of CDK2 alternative splicing by exclusion of exon 5 represses CDK2 protein expression and thus impacts cell cycle control [58]. The regulation of CDK2 alternative splicing is coordinated by poly(rC) binding protein 1 and 2 (PCBP1/2) as well as the splicing factor PTB. PCBP1/2 and PTB promote CDK2 exon 5 inclusion by binding specifically to the C-rich polypyrimidine tract splice acceptor 5′ to exon 5 [58] (Figure 1). Thus PCBP1/2 and PTB promote CDK2 protein stabilization.

Post-translational modifications of splicing factors during cell cycle

Post-translational modifications of splicing factors affect their protein–protein and protein–RNA interactions, intracellular localization, trafficking and activation, thus affecting pre-mRNA splicing as well as alternative splicing. Splicing factor kinases, such as SRSF protein kinase 1 (SRPK1) [59, 60], SRPK2 [61] and CLK1 [26], were shown to change in expression during the cell cycle to phosphorylate their targets in a way that is dependent on cell cycle phase.

Nuclear speckles provide storage for splicing factors with no or few post-translational modifications. Nuclear speckles break down and reform as cells progress through mitosis (Figure 2); this dynamic phenomenon was first shown to be dependent on SRPK1 in HeLa cells [59, 60, 62, 63] and later to be shared with its homologue SRPK2 in various human cells lines and tissues [61]. SRPKs are more active during M phase, leading to nuclear speckle disassembly by phosphorylating splicing factors [64]. Cell cycle-dependent phosphorylation of splicing factors by SRPK1 during M phase was first identified in HeLa cells by the use of a

radioactive isotope of phosphorus (^{32}P) [64]. SRSF1, SRSF2, SRSF3 and SRSF6 splicing factors were shown in HeLa cells to be phosphorylated by SRPK1, but the phosphorylation was not directly connected to cell-cycle regulation. At M phase exit there is reconstitution of the nuclear envelope, and most pre-mRNA splicing factors gradually self-organize to reform the nuclear speckle [62].

One of the first functions discovered for splicing factor phosphorylation during cell cycle was change in activity [11, 65]. SRSF10 serves as a splicing repressor in its unphosphorylated state, while in its phosphorylated state it is a sequence-specific splicing activator [66]. Although PP1 was demonstrated to dephosphorylate SRSF10 following heat shock, it is not known if it is the same phosphatase acting during cell cycle [67].

CLK1 expression level oscillates during the cell cycle promoting auto-phosphorylation that leads to its stabilization and peak expression at G2/M [26, 68]. CLK1's role as a splicing factor kinase [69, 70] is predicted to be the cause of its prominent regulation of alternative splicing during cell cycle and was found to be the central cause of most alternative splicing regulation [26]. Thus, silencing or inhibiting CLK1 leads to late S-phase/G2 phase arrest.

Phosphorylation of the splicing factor SRSF1 was shown in HeLa cells to regulate its function in pre-mRNA splicing [71] and to lead to a change in its subcellular localization in pediatric acute lymphoblastic leukemia cells [72]. In detail, phosphorylation on Tyr-19 by kinase TIE2 allowed cell cycle entry [72]. Furthermore mutating Tyr-19 resulted in cell-cycle arrest in the G0/G1 phase [72], while depleting DT40 cells of SRSF1 led to G2 cell cycle arrest [73]. The M-phase promoting factor, CDK1, phosphorylates SRSF1 on its RS domain on serines 227 and 238 in a cell cycle-dependent manner during G2/M phase [74], while this phosphorylation probably has merit in cell cycle regulation, it has never been demonstrated, nor has SRSF1 phosphorylation by SRPK2 [61, 75].

One of the major splicing factors studied in connection to cell cycle regulation is cell division cycle 5-like protein (CDC5L), also known as CDC5, which is part of the Prp19 complex, a component of the pre-mRNA splicing complex. CDC5L was shown to be essential for pre-mRNA splicing from yeast to humans [76-84]. Since *CDC5L* is expressed constitutively throughout the cell cycle [85], it was suggested that it is regulated via post-translational modification. Indeed it was shown that CDC5L is phosphorylated by CDK2 in a mitogen-dependent manner in monkey COS-7 cells and that its phosphorylation in murine RRC285 cells and human HeLa cells is critical to its association with the spliceosome [86, 87]. Furthermore,

it was found *in vitro* using rat liver nuclear extracts that phosphorylation of CDC5L by CDK2/cyclin E is required for CDC5L binding to the nuclear RNA binding protein, NIPP1 [87] (Figure 1). Depletion of CDC5L causes dramatic mitotic arrest, chromosome misalignments and sustained activation of spindle assembly checkpoint [85, 88-90]. The mechanism can be inferred from data showing that CDC5L modulates the pre-mRNA splicing of a set of genes involved in mitosis [89]. One of these genes is α-*TUBULIN*, which can rescue CDC5L mutations by deleting its intron [91]. This suggests that CDC5L's role as a splicing regulator is needed for proper cell cycle regulation. However, recent evidence suggests that CDC5L has a direct role as a cell cycle component. CDC5L was shown in human HeLa and HCT-116 cells to bind the cell-cycle checkpoint kinase ataxia-telangiectasia and Rad3-related (ATR) and is important for the phosphorylation of its targets such as checkpoint kinase 1(CHK1), cell cycle checkpoint protein Rad 17 (RAD17) and Fanconi anaemia complementation group D2 protein (FANCD2) [92]. Blocking of replication fork in the latter study resulted in cell cycle arrest at the S phase, positioning phosphorylation by CDC5L as a regulator of S phase [92]. It could be that CDC5L regulates cell cycle via splicing regulation in the absence of DNA damage, and in addition has a direct role in cell cycle following DNA damage.

Interestingly the splicing factor CDC40's effect on cell cycle may also be rescued by intron-less α-*TUBULIN*. Saccharomyces cerevisiae *CDC40* encodes a second-step pre-mRNA splicing factor with a role in cell division [93-97]. The transitions from G1/S and G2/M are regulated by *CDC40* as it participates in the splicing of key regulators of cell cycle such as α-*TUBULIN* [98] and *ANC1* [99]. Rescue of cell-cycle arrest in yeast was enabled by genomic replacement with an intronless form of these genes [98, 99].

Change in expression of splicing factors during the cell cycle

Differential expression of a splicing factor in a specific cell cycle phase can alter pre-mRNA splicing to allow for a proper cell cycle progression. Looking at all RNA binding proteins genome wide using RNA-seq, Dominguez *et al*. found 96 RNA binding proteins that oscillate during the cell cycle [26]. The RNA binding proteins were enriched for a function in pre-mRNA splicing and included the RS domain-containing factors SRSF2, SRSF8, TRA2A and SRSF6. These splicing factors were shown to change in expression but it is not clear how they directly regulate cell cycle.

An example of a splicing factor regulated in expression through a cell cycle-dependent manner is Wilms tumor 1-associating protein (WTAP). WTAP protein amount in human umbilical vein

endothelial cells (HUVECs) was found to be low in G1 and increased to reach its peak in G2/M. WTAP high expression in G2/M correlated with that of *CYCLIN A2* and it was shown in HUVECs to stabilize its mRNA through its 3' UTR sequence [100]. WTAP is localized throughout the nucleoplasm as well as in the nuclear speckles and partially colocalizes with splicing factors in HUVECs [101]. WTAP also binds to the RNA processing factors THRAP3 and BCLAF1 and this binding was shown to be critical to its localization to nuclear speckles [101]. Interestingly, THRAP3 and BCLAF1 regulate pre-mRNA splicing of genes involved in cell cycle regulation [102].

Another splicing factor with differential expression in cell cycle is RBM42 (TgRRM1) [103]. RBM42 was identified in the *Toxoplasma* parasite and its expression was found to be highest in late M and early G1. RBM42 binds the spliceosome components snRNA U4, U5 and U6 and is critical to the pre-mRNA splicing process. A point mutation in RBM42 in *Toxoplasma* led to G1 arrest with cell cycle regulation by RBM42 speculated to occur due to its role as a pre-mRNA splicing factor [103].

Chromatin alteration during cell cycle and its effect on alternative splicing

Recent evidence points to a contribution of epigenetic marks and higher order chromatin structure to alternative splicing regulation [104]. Moreover, genome-wide mapping has revealed enrichment of nucleosomes in exons [105, 106]. In addition, several histone modifications are enriched in exons relative to introns and numerous modifications, such as H3K36me3, are differentially associated with constitutive exons relative to alternative ones [107]. Since chromatin is dynamic during the cell cycle, it is possible that it serves as a regulator of alternative splicing.

The three histone modifications H3K36me3, H3K27ac, and H4K8ac mark alternatively spliced exons [108]. This was observed by following chromatin and alternative splicing states in human embryonic stem cells relative to differentiated cells. More than half of alternative splicing genes regulated by H3K36me3, H3K27ac, and H4K8ac are enriched for genes with function in G2/M cell-cycle phases [108]. Interestingly the effect of the histone modifications on alternative splicing was suggested to be mediated via transcription factors with a known role in cell differentiation [108].

A recent study identified the **histone demethylase** KDM3A as a regulator of alternative splicing during cell cycle [109]. RNA-seq following silencing of KDM3A revealed a group of genes with a role in cell cycle. Interestingly, KDM3A is a regulator of both transcription and

alternative splicing, but acting independently, as the expression and alternative splicing genes formed two separate groups. KDM3A was found to be necessary for the binding of the splicing factor SRSF3 to pre-mRNA. Silencing either KDM3A or SRSF3 promoted a higher percentage of cells in S-phase. Furthermore, this study indicated that alternative splicing of a target of KDM3A, the cell cycle gene *SAT1*, is the mediator between KDM3A and cell cycle regulation. Finally, these findings suggest that change in chromatin during the cell cycle can promote regulation of alternative splicing of genes important to this process.

SF3B1 and its splicing inhibitors role in cell cycle

The spliceosome component SF3B1 binds and stabilizes the **small nuclear RNA (snRNA)** U2. U2 binding to the branch point site is important for splice site recognition [110, 111]. The binding of the complex bulges out the adenosine at the branch point to facilitate the first catalytic reaction in the splicing cycle [112]. In addition, SF3B1 allows the identification of short exons flanked by long introns with differential GC content by binding to the exons' **nucleosomes** [113] (Figure 3). Both functions of SF3B1 rely on its phosphorylation at several sites. SF3B1 phosphorylation was also found to be cell cycle dependent. One SF3B1 kinase identified is dual-specificity tyrosine-phosphorylation regulated kinase 1A (DYRK1A), which phosphorylates it on its Thr434 site [114, 115]. In addition, the G1 phase complex CDK2/cyclin E phosphorylates SF3B1on Thr313 and this specific phosphorylation was shown to be critical for active splicing [116]. Many more possible phosphorylation sites are located mainly at its N terminal domain. Murthy *et al*. demonstrated cell cycle-dependent phosphorylation of SF3B1 [117]. This study suggested that nucleosome binding of SF3B1 is dynamic as a result of its phosphorylation and thus regulates alternative splicing of cell cycle genes. In detail, SF3B1 phosphorylation peaks at G2/M in a CDK1-dependent manner, and then decreases due to PP2A and PP1 phosphatase activities, as the cell progresses through G1/S [117] (Figure 3). Notably, SF3B1 binding to nucleosomes increases during G1/S when it has the lowest phosphorylation level. In a complementary manner, the interaction with nucleosomes is greatly diminished during G2/M, when the phosphorylation level is higher [117] (Figure 3).

The link of SF3B1 to cell cycle progression gained attention due to the elevated levels of *SF3B1* mutants in several types of cancers such as myelodysplastic syndrome (MDS), chronic lymphocytic leukemia, uveal and breast cancer [118-121]. How these mutations result in cancer is not yet fully understood. However, a few studies have shown that *SF3B1* mutations lead to the use of cryptic 3`-splice sites upstream of the canonical one and to the promotion of the selection of an alternative branch point [122-125]. Kesarwani *et al*. showed in human

transfected K562 cells and in cells derived from MDS patients, that these cryptic splice sites are detectable by the spliceosome because of a structural change that suffers upon the inclusion of a mutated SF3B1 subunit [126]. The effect of these events is an altered splicing pattern of a large list of genes related to cell cycle [127, 128]. In another study the disruption of SF3B1 induced a significant downregulation of pathways related to cell cycle regulation and led to G1 and G2 arrest in human MDS stem and progenitor cell lines (TF1, K562, HEL and SKM1) [129].

Huang *et al*. showed that the knockdown of SF3B1 in human erythropoietic cells caused early cell cycle arrest by the activation of the p53 pathway due to an isoform switch of E3 ligase makorin ring finger protein 1 (MKRN1) and downregulation of genes involved in mitosis and cytokinesis [130]. In loss-of-function zebrafish mutants, erythrocytes also presented with G0/G1 arrest [131]. Here, SF3B1's effect was suggested to cause elevated expression of transforming growth factor β (TGFβ) and decreased expression of cell cycle genes [131].

A significant tool to study the role of SF3B1 in cell cycle are its many inhibitors (reviewed in [132]). All SF3B1 inhibitors studied have demonstrated anti-tumoral properties [110, 133-136] and a common pharmacophore model by which their specific interaction with SF3B1 provokes a conformational change and leads to splicing alteration due to a branch point recognition disturbance [111, 132, 137]. The fact that cells with SF3B1 inhibition or downregulation leads to similar aberrant splicing patterns suggest that affected genes share similarities at the 3`splice site and weak branch point sites [126-128, 132]. Interestingly each of the SF3B1 inhibitors can have a different effect on cell cycle [138]. Pladienolide B can lead to early (G1) as well as late (G2/M) arrest in two erythroleukemia cell lines, HEL and K562 [139], while isoginkgetin provokes slowdown of multiple phases in the cell cycle (G1, S and G2) but not M phase in human colon HCT116 cells and ovarian cancer A2780 cells [140]. A detailed investigation of the effect of spliceostatin A found reduced cell proliferation in HeLA cells due to decreased expression of cell cycle genes such as *CYCLIN A2* and *AURORA A KINASE* [111]. The mechanism that leads to the change in expression was suggested to be mediated by nonproductive pre-mRNA splicing. In a study using four types of human cells, HeLa, Rh18, HEK293 and primary fibroblasts, it was found that Sudemycin E inhibits SF3B1 binding to nucleosomes. Diminished H3K36me3 mark in these nucleosomes was also detected and cell cycle arrest was observed (G2 phase) [141]. We have described here various cell cycle alterations by SF3B1; these diverse and sometimes contradicting effects might stem from its differential expression in different cell types with diverse genetic backgrounds [142, 143].

Concluding Remarks

There is an increasing awareness of the importance and potential of pre-mRNA splicing in disease and therapy. Furthermore, alternative splicing is of great physiological relevance as it enables the production of multiple protein isoforms from a single pre-mRNA molecule by the combinatorial use of splice sites. The various protein isoforms produced can have different functions and as such alternative splicing contributes significantly to regulation of cellular function. Here we focus on the novel, and very exciting, insights into the emerging field of splicing modulation during the cell cycle.

The exact splicing patterns in each of the cell cycle phases is yet to be rigorously characterized. In the future combining this data with the different levels of alternative splicing regulation described here, namely splicing factor expression; post-translational modification and chromatin dynamics, can set the path to a deeper understanding of alternative splicing regulation in cell cycle and beyond (see Outstanding Questions box).

Figure Legends

Figure 1: Alternative splicing of CDK2 is regulated in order to phosphorylate splicing factors important for cell cycle progression. A. PCBP1/2 and PTB bind the 5' splice site of CDK2 exon 5 (dark red rectangle) to promote its inclusion. CDK2 exon 5 inclusion gives rise to a more stable protein serving as a more potent kinase. B. The CDK2/cyclin E complex phosphorylates retinoblastoma (RB) protein to drive the cell to S phase. In late S phase CDK2/cyclin A phosphorylates CDC6 to allow for exit from S phase. Finally CDK2/cyclin E phosphorylates the splicing factor CDC5L, which regulates the splicing of many genes involved in cell cycle.

Figure 2: Nuclear speckles are stable during interphase and disperse during early metaphase. Cellular localization of the nuclear speckle marker SRSF2 in HeLa cells at different cell cycle stages. Arrow heads denote splicing speckles. Scale bar 6.5 μm.

Figure 3: The spliceosome component SF3B1 is essential for cell cycle progression. SF3B1 phosphorylation peaks at G2/M in a CDK1-dependent manner, and then decreases due to PP2A and PP1 phosphatase activities as the cell progresses through G1/S. SF3B1 in its unphosphorylated state allows the identification of short exons flanked by long introns by binding to the exons' nucleosome. SF3B1 binding to nucleosomes allow for alternative splicing of cell cycle genes.

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Glossary

Chromosomal passenger complex (CPC): This functions at the centromere to ensure correct chromosome alignment and segregation.

Enhancer RNA: short non-coding RNA molecules (50-2000 nucleotides) transcribed from the DNA sequence of enhancer regions which have a role in transcriptional regulation.

Histone demethylase: An enzyme that removes methyl (CH3-) groups from histones that is important in epigenetic modification mechanisms. Histone demethylases alter transcriptional regulation of the genome by controlling the methylation levels that occur on histones and so regulate the chromatin state at specific gene loci within organisms.

Nuclear speckles: nuclear domains enriched in pre-mRNA splicing factors, located in the interchromatin regions of the nucleoplasm of mammalian cells.

Nucleosomes: the basic structural unit of DNA packaging in eukaryotes consisting of a segment of DNA wound around eight histone proteins. It is the fundamental subunit of chromatin.

R loop: a three-stranded nucleic acid structure, composed of a DNA:RNA hybrid and the associated non-template single-stranded DNA, formed in a variety of circumstances. The presence of R-loops can inhibit transcription and R-loop formation appears to be associated with “open” chromatin, characteristic of actively transcribed regions.

Small nuclear RNA (snRNA): a class of small RNA molecules of around 150 nucleotides that are found within the splicing speckles and Cajal bodies of the cell nucleus in eukaryotic cells. Their primary function is in the processing of pre-messenger RNA (hnRNA) in the nucleus.

Unstructured domains: also called auxiliary domains, these are proline-, glycine- or acid-rich. These domains are speculated to provide the specificity of the RNA binding domain in the hnRNP protein family. This is facilitated by the 3D structure of the protein around the RNA binding domains which fine-tunes the RNA–protein interaction.